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Influence of environmental and genetic factors on food protein quality: current knowledge and future directions

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Dietary protein quality is commonly defined by the bioavailability of essential amino acids, a function of amino acid composition and protein digestibility. This review assesses the potential for manipulation of amino acid composition in organisms, for improving protein quality in nutrition. Animal protein is generally regarded as higher quality than plant protein, but it is also relatively resistant to change. Plant protein quality appears more susceptible to genetic and environmental influence with seed storage protein a potentially promising target, subject to GMO regulatory limitations. There is increasing interest in alternative dietary-protein sources including insects and fungi or other microorganisms. Each may be manipulated through environment or diet. Microorganisms also enable assessment of impacts on protein quality of biochemical-pathway manipulation or tailored growth regimes. We conclude that such approaches offer the greatest potential for manipulation. These means could help in producing protein of sufficient quantity and quality to meet future demand.

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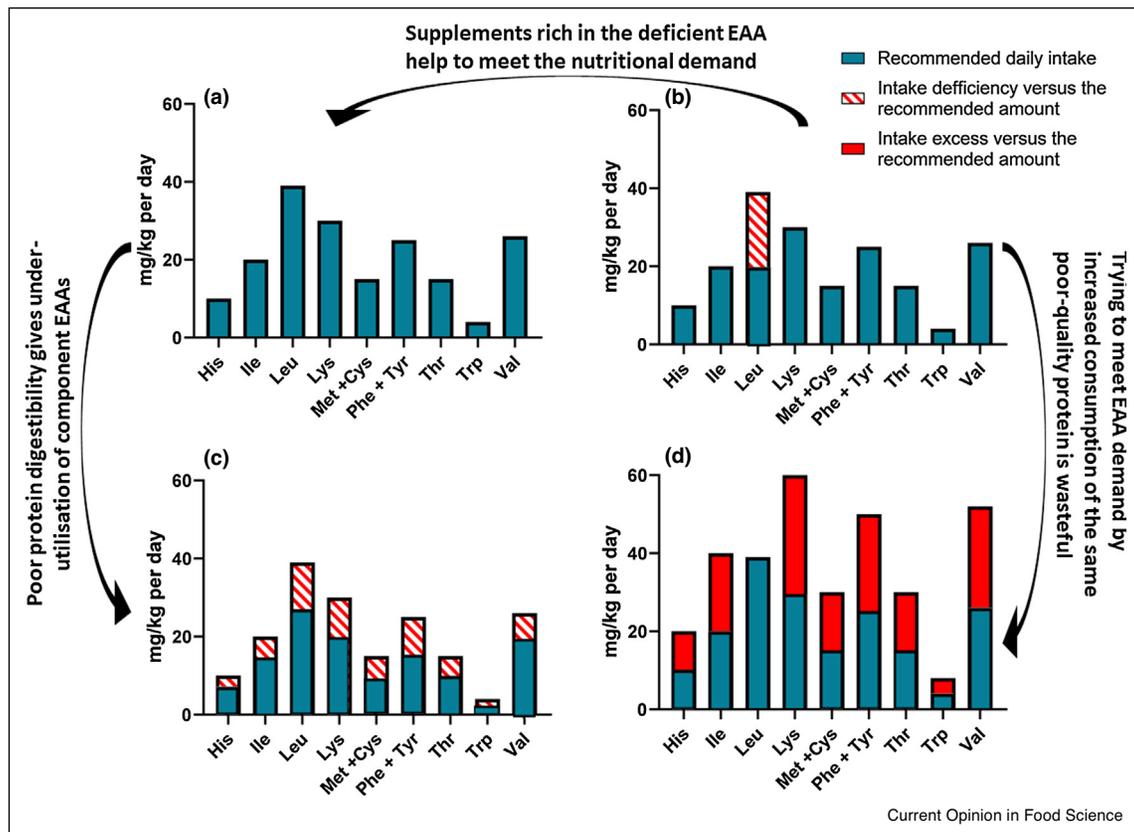
Introduction

There is increasing concern over our ability to produce sufficient food sustainability for the growing global population. There are large variations in both the amount and source of dietary protein consumed by different populations, but reliance on animal-based protein

systems is widely regarded as unsustainable [1]. Protein malnutrition in developing countries, resulting in impaired physical and mental development, remains an ongoing problem [2]. In developed countries, many individuals consume considerably more protein than required to maintain health and some evidence suggests this could contribute to high incidence of obesity, diabetes and related conditions [3]. High quality protein is characterised by an appropriate balance of essential amino acids (EAAs), good digestibility and absence of anti-nutritional factors (e.g. trypsin inhibitors in unprocessed legumes or uricogenic nucleobases in certain microorganisms) [4,5]. An ideal protein source meets EAA requirements without further supplementation. According to the WHO-preferred method of amino acid profile evaluation (PDCAAS; the protein digestibility-corrected amino acid score) protein sources scoring close to ideal include eggs and milk [6]. Figure 1 illustrates how different protein-quality scenarios can affect dietary EAA supply. Protein requirements are also impacted on by physiological state, with increased requirements in pregnancy, lactation, childhood growth and in elderly people [6]. Whereas the daily recommended intake of high-quality protein for young adults is 0.8 g/kg/day, for elderly adults, whose numbers are increasing in many developed countries, 1.2–2 g/kg/day is recommended to help slow the loss of muscle mass known as sarcopenia [7].

The amino acid (AA) composition of a specific protein is governed by the nucleic acid sequence of the gene which codes for it. In animals, all proteins have specific physiological/metabolic functions. In plants, additional proteins may be produced for storage within seeds [8]. As such, manipulation of the AA composition of animal and plant-based proteins is largely dependent on altering the relative amounts of different proteins associated with the tissue to be consumed. While these principles also apply to microorganisms, versatile selection methods that avoid genetically modified organisms (GMOs) may allow us to alter the AA composition of specific microbial proteins (without necessarily impairing function or organism fitness, as discussed further below) or even facilitate production of novel proteins [9–11]. This article provides examples of environmental and genetic factors which impact protein composition and discusses how these might be exploited to produce higher quality protein. Focus is on factors which may have especial impact on amounts of different AAs rather than digestibility.

Figure 1



Potential protein-quality scenarios and effects on dietary essential amino acid (EAA) supply. **(a)** EAA profile (x axis) of 60 g of a protein source of an ideal quality [6]. **(b)** EAA profile of a near ideal protein source deficient in Leu. **(c)** Poorly digestible protein or protein containing antinutritional compounds may not be completely utilised despite having a good EAA profile **(d)** Consuming more poor-quality protein to compensate for particular deficiencies (b) leads to excess consumption of EAAs that are already available in sufficient amounts.

Animal protein

Extent of variability in animal protein

As described above, the AA composition of animal products is largely governed by the biological function of the product. Thus, eggs are governed by the needs of the developing fetus, milk protein by the AA requirements of offspring and meat by muscle function requirements. However, a number of effects of animal age, species or diet have been described.

Rafiq *et al.* [12] determined the amount and AA composition of the major proteins (caseins and whey) in the most commonly consumed milks. Casein was the predominant protein in all milks but AA composition varied significantly between species. There is evidence that the total protein content of cow's milk can be altered by feeding different diets [13], but other work suggests this has limited impact on the relative amounts of different proteins and, therefore, the AA composition [14]. Hen's eggs provide high quality dietary protein but the limited data

available suggests their AA composition is not significantly affected by the breed of bird or by altering the protein content of their diet [15].

Meat is another major source of animal protein in human diets, the most commonly eaten types being chicken, pork and beef. Since the mid-twentieth century, genetic selection and improvements in nutrition and environmental conditions have dramatically increased growth and muscle mass in livestock, particularly poultry [16]. However, some evidence indicates this may have unfavourably impacted AA composition. For example, genetic selection for ~5% increased broiler breast-meat mass between 2001 and 2012 was associated with increased incidence of wooden breast (WB) and white stripes (WS) myopathies, which are thought to result from insufficient oxygenation of rapidly growing muscle, among other causes [17]. Affected poultry have lower protein quality with the most affected meat showing significantly decreased levels in 8 of 10 EAAs [18] (Table 1).

Table 1

Factors effecting particular changes to food protein EAA profiles			
Food source	Factor	Effect	Reference
Broiler chicken	Wooden stripes and wooden breast myopathy	Significant decreases in EAAs compared to unaffected meat, per unit meat weight: Arg (41%); Ile (16%); Leu (13%); Lys (24%); Met (9%); Phe (15%); Thr (11%)	[18]
Pig	Supplementing feed with Chinese herbs mixes	Significant increases in EAAs per unit meat weight compared to basal feed: Arg (3.5%); Met (42%); Leu (6%); Ile (6%); Phe (6%)	[21]
Insect larvae (beetle; <i>Protaetia brevitarsis</i>)	Supplementing basal feed (fermented sawdust) with plant materials	Significant increases in EAAs relative to larva weight when supplementing either with apple (Met, 35%; Phe, 7%), aloe (Met, 30%) or sweet persimmon (Phe, 3%)	[27]
Potato (<i>Solanum tuberosum</i>)	Replacing conventional fertiliser by organic fertiliser	Significant increase in EAAs compared to control, per unit dry weight: Arg (48%); Ile (42%); Leu (106%); Trp (50%); Val (79%)	[39]
Butternut squash (<i>Cucurbita moschata</i>)	Replacing conventional fertiliser by organic fertiliser	Significant increase in EAAs compared to control, per unit dry weight: Arg (26%); His (39%); Lys (25%); Ile (47%); Phe (76%)	[40]
Wheat grains	Growing in soil containing metal compound nanoparticles	Significant change in grain AA contents, compared to control, when exposed to nanoparticles comprising: Fe ₂ O ₃ (Tyr, +20%); CuO (decreased Leu, His, Thr); Ag (concentration-dependent decreases in His, Asp, Glu, Leu, Ile and in total protein); CeO ₂ (Arg +21.6%, Lys +15.8%, Gly +14.1%, His +16.2%, with no significant change in total AA content).	[43–45]
Onion	Long term storage	After storage for 5 months at 2–3°C: significant decreases in Leu and Ile but significant increases in Met, Cys, Phe, compared to fresh bulbs After storage for 9 months at 20–25°C: general decrease in EAA levels compared with fresh bulbs	[64] [65]
GM maize LY038 strain	Increased production of lysine	Wild type maize: Lys in protein (2.55 mg/g); free Lys (0.05)	[36]
<i>Fusarium venenatum</i> (fungus)	Changing sugar type in growth medium	LY038: Lys in protein (3.70); free Lys (0.96) Approximate 20% decrease in total amino acid content when grown on ribose versus glucose	[50]
<i>Lactococcus lactis</i> (bacterium)	Selection for AA over secretion mutants	Isolated mutants secreted more AAs (mM) versus wild type in mid exponential growth phase (ND, not detected). Wild type: Glu (5); His (3); Val (2); Met (ND); Ile (ND); Leu (ND). MUT-15: Glu (50); His (6.8); Val (22); Met (ND); Ile (2); Leu (22). MUT-91: Glu (48); His (6.5); Val (24); Met (ND); Ile (2.5); Leu (30). MUT-54: Glu (35); His (6); Val (16); Met (1.4); Ile (1.1); Leu (16).	[10]

Elsewhere, minor differences in EAA profile of poultry, cattle or pigs have been recorded variously between animal sexes, between parts of the carcass, from dietary effects or regional variation in these or other parameters [19–21]. In fish, differences in AA profile have been recorded between species including from different habitats, observations that could also partly reflect dietary differences [22].

Overall, however, traditional animal protein seems to offer relatively limited opportunity for EAA manipulation for human benefit, especially as some conditions described above come with disadvantages.

Challenges and opportunities in optimising insect protein

Insects are an important dietary protein source in many parts of the world but have not yet gained widespread popularity in Western diets. It was only in 2018 that the EU approved whole insects, or their parts as novel foods. There is also growing interest in the use of insects as feed

for farmed animals and fish [23]. Insect protein is typically similar quality as traditional livestock protein, but insects are relatively easy and quick to grow, consume less water and emit less CO₂ [24]. Besides species–species differences, insect protein quantity and quality is subject to factors such as gender, temperature, daylight duration and feed type [25,26]. It is noted that most insects are analysed whole and, as such, the gut contents may make a significant contribution to protein content. EAA profiling showed that switching feed from alfalfa to maize for edible grasshoppers produced 40% decreases in levels of histidine and phenylalanine per gram of protein [25]. Similar analyses with larvae of the *Protaetia brevitarsis* beetle revealed a modulating influence of supplementing the base larval feed on the absolute levels of some EAAs, compared to non-supplemented control feed. The methionine level was increased by ~35% or ~30% in feeds supplemented with apple or aloe, respectively, and phenylalanine by ~7% or ~3% in feeds supplemented with aloe or sweet persimmon [27]. It is worth noting, however, that these supplemented feeds also resulted in decreased

overall protein quantity in inspected larvae. Knowledge of such relationships potentially allows producers to improve protein quality by appropriate feed supplementation. However, this versatility needs to be balanced against possible downsides of a high fat/protein ratio with some insect feeds. For example in black soldier fly larvae, a potential alternative fish meal, the high variability of final product raises concerns about economic viability [28].

Plant protein

Plants as protein sources

EAA contents of plant proteins are generally lower than those of animal proteins [29]. Whey, muscle and milk proteins have EAA contents between 38–43%, whereas oat, lupin and wheat proteins have EAA contents between 21–22% [5]. A plant-based diet can provide all of the EAAs but requires a relatively rich variety of fruit and vegetables or preparation as a blend of plant proteins, either of which can be hard to access in some regions [5,16]. However, the growing market for plant-based meat substitutes offers a convenient vehicle to deliver such blends [30]. Additional opportunities may arise from crops that are currently underutilised (e.g. particular legumes) and which may be native to specific regions [31]. Another issue is that some protein rich plants have low digestibility and/or contain antinutritional factors [4]. Extensive processing is often required to address this. Nevertheless, increased consumption of plant protein is incentivised from a sustainability perspective, besides considerations like animal welfare. Currently, a portion of high value crops like soya, wheat and maize are used as livestock feed, where 3–6 MJ of plant protein that is edible for humans may only produce 1 MJ of meat protein [16,32]. Therefore, from a resource-use perspective, there are key advantages to improving crop quality for direct human consumption rather than increasing meat production.

EAA enrichment of seed storage protein

As protein sources for the human diet, legumes suffer from deficiencies in the EAAs Lys and Met, and cereals from deficiencies in Lys, Met and Trp. Consequently, there has been considerable effort using both traditional breeding and GM approaches to produce cultivars with increased amounts of these EAAs [8,33]. In terms of protein for human consumption, seed storage protein has shown the most promise for EAA enrichment as seeds are relatively insensitive to accumulation of (either native or non-native) storage protein [8]. That is, storage protein of seeds offers better opportunity for non-detrimental manipulation of content than is available with protein from animals or vegetative plant tissue. The ‘Quality protein maize’ project, developed through selective breeding approaches focused on control and biosynthesis of seed storage proteins in maize endosperm, yielded product during the 1990s that contained approximately

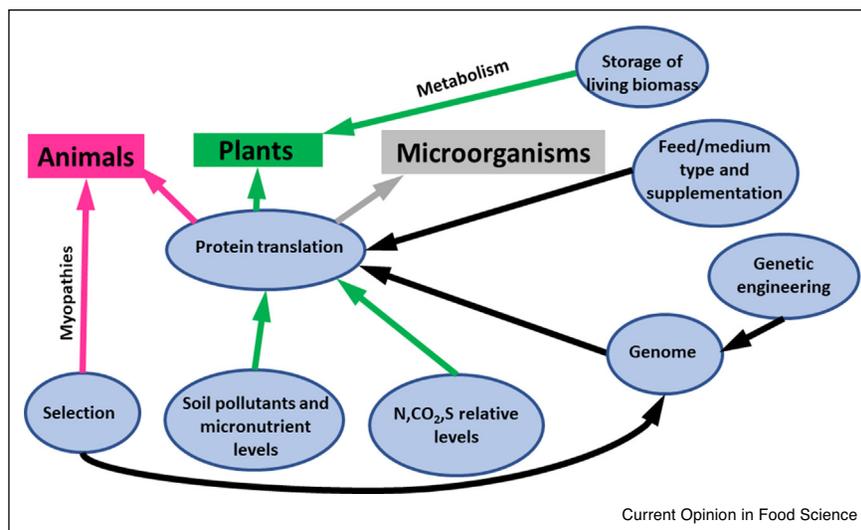
twice the lysine content of traditional maize [8,34]. The derived maize strain has been commercialized and used in many countries. However, success with these breeding approaches is limited as increased Met, Lys and Trp phenotypes often have deleterious effects on growth. This can be because the genes yielding increased content of these EAAs are not regulated in a seed specific manner. This is also reflected by poor success in attempts to replicate the quality protein maize effect in other crops [8]. Therefore, instead there has been emphasis on seed-specific manipulation of AA synthesis; for example, desensitization to end product inhibition or altered expression of proteins with particular EAA contents [8,33]. Approaches used to increase Met content in crops involved increasing or decreasing, respectively, the expression of Met-rich or Met-scarce seed storage proteins, or introduction of foreign Met-rich proteins; however, the resulting varieties exhibited growth defects [8]. A high Lys maize genotype, LY038, was produced by embryo-specific expression of feedback-insensitive dihydrodipicolinate synthase from bacteria [35]. This was approved for commercial use as livestock feed and shown to be superior for broilers compared to the wild type maize [36]. However, LY038 was later withdrawn reportedly due to human safety concerns raised by the European Food Safety Agency, even though it was intended for use as animal feed. The size of the EU market means that its laws affect use not only of its crops but also that of producers wishing to trade with the EU [37]. The overall potential for improvement of crop nutritional quality with GM approaches is well understood but GM food regulations hamper application.

Influence of cultivation conditions on AA profiles of plant crops

There are some conflicting reports on the superiority or inferiority of organically grown fruits and vegetables with regard to protein quality [38]. However, several studies now suggest that organic fertilisation can improve the protein quality. Potatoes and butternut squash exhibited small but significant improvements in total EAA levels when grown with organic fertiliser [39,40] (Table 1). Such increases have been suggested to reflect differences in nitrogen availability through growth [39].

Other factors also influence protein quality in plants (Figure 2 summarises a range of factors relevant to plants, animals or microorganisms). Recent evidence suggests that relative nitrogen to sulphur availability may modulate expression of AA-synthesis genes in wheat (*Triticum monococcum*) [41]. Elsewhere, meta-analysis of the effects of elevated CO₂ indicated decreased plant-protein contents (as well as decreases in certain elemental contents, e.g., S, Fe, Zn) [42]. Metal nanoparticles (MNPs) in pure form or their compounds have diverse applications (including in fertilisers and pesticides) and are becoming more prevalent in soil and water bodies. A number of

Figure 2



Factors linked to changes in protein AA composition. Simplified schematic of interacting factors that may influence protein AA composition. Arrows indicate reported effect either on protein AA composition of the indicated organisms (organism groupings distinguished by pink, green or grey; or black if applicable to more than one grouping) or on another of the factors shown.

studies have documented that exposure to MNPs can negatively affect AA contents of plants, but in some cases increases were also observed [43–45] (Table 1). Nevertheless, any suggested exploitation of such insight would of course be subject to regulatory constraints around using toxic MNPs in crop cultivation.

In conclusion, for similar reasons as with animal tissues, plant tissues appear to have limited potential for manipulation of EAA composition. One exception is seed storage protein (where functional protein is less important for the organism) that has significant potential for improved protein quality and application, supported by data. This potential though is subject to GMO regulations.

Microbial protein

Use of single cell protein as a protein source for humans

Single cell protein (SCP) describes protein originating from microorganisms, both unicellular (e.g. yeast, bacteria) or multicellular (e.g. filamentous fungi, algae) [46]. These could potentially be principal protein sources in everyday diets that integrate different protein components, including plant-based. Despite the idea being decades-old, SCP has historically been used as a supplement or animal feed; for example, MarmiteTM and PruteenTM. Mycoprotein from the filamentous fungus *Fusarium venenatum* and marketed as QuornTM was first sold as a meat-substitute in the UK in 1985, and has been the only SCP sold for human consumption [47]. Recently other companies have also started to launch SCP products for this market. Bacterial and fungal SCP contains

between 80–90% and 50–60% protein by dry mass, respectively, with EAA profiles comparable to those of animal protein. The methionine content in fungi tends to be lower but is within dietary guidelines [46]. SCP production has some unique challenges, as fungi and bacteria contain high levels of nucleic acids (7–12%) that need to be lowered by additional steps in production [48]. There is also the risk of toxin production by the organism, absence of which needs to be routinely tested [47]. This could also bring challenges for modifying the SCP production process, as changing the growth substrate or other condition might activate toxin production [49]. Benefits of SCP over traditional animal protein include lower carbon footprint, land use and water consumption, and the potential to use industrial food by-products as growth substrate [48,49]. However, to date SCPs for human consumption are grown using food grade substrates, with associated costs [47]. Wider adoption of SCP for human consumption not only promises potentially cheaper, sustainable protein production but also scope to modify the protein composition of target organisms, which in plants and animals could be too time consuming, expensive or in some cases unethical.

Relative simplicity and short generation times provide unique ways to improve SCP quality

Because of their fast cell-doubling times, fungi or bacteria can be selected over hundreds or thousands of generations in weeks or months, in marked contrast to most animals and plants. Thus, adaptive evolution is often used for strain improvement and this avoids use of genetic engineering and its attendant restrictions for food

purposes. Knowledge of metabolite biosynthesis pathways in fungi and bacteria provides additional opportunities for targeted manipulation of AA profiles. Microbial strains with specific AA production features can be isolated through selection screens. For example, culturing yeast with 5,5,5-trifluoro-DL-leucine (TFL) — a non-metabolised leucine analogue — can select cells that overproduce leucine due to loss of feedback inhibition of leucine production [11]. Other approaches may not require targeted manipulation of specific biosynthesis pathways. Simple changes in sugar source can alter the AA content of *Fusarium* species [50]. Continuous adaptive selection was used to find mutants of the bacterium *Corynebacterium glutamicum* (which is used for industrial AA production) that could grow rapidly without the need for addition of expensive growth-boosting additives [9]. Biosensors can be developed for high throughput screening and selection of mutants such as overproducers of particular AAs [10] (Table 1). Similar strategies could be used to improve SCP production efficiency; for example, by improved growth on a waste feedstock. Such approaches lend themselves to screening large numbers of strains relatively cheaply and quickly, enabling selection of organisms with desirable nutritional properties without the need for genetic engineering.

Using different visible-light wavelengths to modify microalgal AA synthesis

SCP from algae has a high protein content (up to 70%), the organisms containing relatively low levels of nucleic acids (3–8%) and grown typically via photosynthesis [46]. Currently algal SCP is mainly used only as a supplement because of its relatively high production costs. However, work to lower these costs may help expand algal use from a supplement to primary protein source [51]. *Spirulina* spp. are algae of especial interest for SCP because of their high protein content and complete EAA profile [52]. The use of LED lamps over fluorescent lamps for photosynthetic growth improves *Spirulina* SCP production-efficiency due to lower light source costs and a near two-fold reported increase in protein yield [53]. Moreover, the use of different wavelengths or comparison of full versus partial illumination gave altered levels of individual free-AAAs, with algae grown under green LED light having the highest level of free AA (~225% increase per g biomass versus the control under fluorescent light) [53]. This may reflect demand for complex nitrogen compounds during photosynthesis, using the free AAs as primary building blocks. These effects of light wavelength could offer relatively inexpensive options for manipulating AA levels in cultivated products and potential tailoring for human or livestock feed.

Can the process of protein translation be manipulated for improving SCP?

The AA composition of proteins is determined by sequence encoded in organisms' genomes. During

protein synthesis, the relevant DNA sequence is first transcribed into mRNA, which serves as a template for ribosomes to link individual AAs that are carried by tRNA molecules. Each tRNA molecule has an anticodon that matches a codon sequence on the mRNA strand to an AA-specific for that anticodon. However, this process of mRNA translation is not error free, creating potential for some variability in the AA composition of synthesised proteins. Translation errors arise primarily during either tRNA aminoacylation, where an AA may associate with the incorrect tRNA molecule, or polypeptide chain formation where an mRNA-codon:tRNA-anticodon mismatch is accepted by the ribosome [54,55]. Translation error rates (once every $\sim 10^3$ – 10^4 codons) are higher than error rates in DNA replication (every 10^9 – 10^{10} nucleotides) or mRNA transcription (every 10^4 – 10^5 bp) [56]. Translation accuracy (hence fidelity of protein-AA composition) varies between organisms and is influenced by factors including translation rate, proof-reading enzyme activity and environmental triggers such as oxidative or starvation stress [57–59]. AA misincorporation, where an AA different to that encoded by the mRNA is introduced to the growing AA chain, is usually considered deleterious because it may cause protein misfolding and loss or change of function, including in essential proteins [54]. However, misincorporation can also provide a tool for adaptation, with organisms tolerating or sometimes benefitting from it [60]. The yeast *Candida albicans* can show up to 28% misincorporation of leucine in place of serine with beneficial consequences for its fungus-host interactions, for example [61]. Furthermore, global misincorporation patterns can be mapped and predicted to some extent. For example, hamster ovarian cells grown in medium limited for one EAA and providing an abundance of others showed distinct misincorporation propensities [55,57]. AAs near-cognate to the deficient AA were most likely to be misincorporated. There could be potential to harness this growing understanding in tailoring quality of protein-products for food, as it becomes more apparent that an ideal human diet can be person-specific [62]. Attempts to modify protein product by manipulating translation are not without precedent. For example, expression of a mutant tRNA in rice enabled introduction of Lys at alternative codons and Lys enrichment in seed storage proteins [63]. Further research on the potential for manipulating translation to yield more 'AA versatile' SCP sources could offer one means to help support personalised diets of the future.

Concluding comments

It is apparent that, while AA composition does differ between animal species and gender, the limited evidence available suggests lesser effects of diet. However, it should be remembered that all animal sources of protein contain an appropriate mix of highly digestible EAA, and as such, populations with free access to such products are unlikely to suffer AA deficiencies. By contrast,

populations dependent on plant sources of protein, particularly cereal crops, are much more susceptible. Hence the ability to manipulate AA composition of plants, and other non-animal sources, could have a major impact in reducing the incidence of EAA deficiency. The scope for genetic manipulation in plant seed protein and potentially other plant parts is reasonable, however current GM food laws make these types of crops largely unusable commercially. The current potential for manipulation of EAA in SCP is higher due to more versatile selection methods that can circumvent the need for genetic engineering. A variety of factors with smaller effects on protein composition is only beginning to be understood (Table 1). Research to date highlights the complex network of effects that can regulate and ultimately alter protein quality, from the level of translation through to whole organism (Figure 2). It is clear that our livestock-reliant food system operates unsustainably but it is also unrealistic to expect a sudden change to the way in which food is produced globally. Therefore, it is important both to introduce more sustainable protein sources and, in parallel, to improve protein-quality and reduce wastefulness in existing food systems.

Declaration of interest

None.

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